Commentary

Another role highlighted for estrogens in the male: Sexual behavior

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Models of lack of estrogen representation, whether they be failure of synthesis or insensitivity of response, have provided new, and in some respects counterintuitive, insights into the roles of estrogens in both males and females. Among the natural mutations, there is currently 1 man identified with a mutation of the estrogen receptor α (ERα) and up to 10 individuals with mutations of the gene encoding aromatase, the enzyme responsible for estrogen biosynthesis. Of these, 2 are men. In addition, there are various mouse models involving targeted gene disruption. These include knock-outs of the ERα (αERKO; ref. 1) and ERβ (βERKO; ref. 2) genes as well as the double ERα and ERβ ($\alpha\beta$ERKO; ref. 3) knockout, in addition to the aromatase knockout (ArKO; refs. 4–6) mice. Analysis of the phenotypes resulting from these mutations has revealed various degrees and types of infertility in both male and female, depending on the mutation involved. Lipid and carbohydrate phenotypes involving increased adiposity with insulin resistance, hyperlipidemia and hyperleptinemia as well as disturbances in behavior patterns, both social and sexual, have also been described (6–8). These studies reveal that estrogens have more extensive roles in physiology than was previously thought, and moreover these are frequently of a non-sexually dimorphic nature. Indeed the traditional concepts of the terms *estrogen* and *androgen* are now challenged because the role of estradiol in the regulation of spermatogenesis (3, 9) is one that would more properly be classified as androgenic.

Another important concept that has emerged in recent years is that in postmenopausal women, as well as in men, estrogens function primarily as paracrine or even intracrine factors (10, 11). Thus, following menopause, when the ovaries cease to produce estradiol and circulating estrogen levels are extremely low, estrogen is produced in a variety of extragonadal sites and acts locally to stimulate adjacent cells or even the cells in which it is produced. These sites include osteoblasts and chondrocytes of bone (12), where it plays a key role in the maintenance of bone mineralization in both males and females; adipose tissue, particularly of the breast, which is the major site of formation of estrogen driving breast cancer development in the postmenopausal woman (reviewed in ref. 13); and also numerous sites in the brain, where it serves key roles in the regulation of reproduction, behavior, and perhaps also cognitive function.

Both estrogen receptors, namely ERα and ERβ, which bind to estradiol with similar affinity, have been identified in numerous sites in the brain (14, 15). For example, both are present in the arcuate nucleus and the preoptic area of the hypothalamus, while ERα is present in the ventromedial nucleus and ERβ in the paraventricular nucleus. These regions of the hypothalamus are important in reproduction, sexual behavior, thermoregulation, and feeding behavior. Both receptors also appear to be present in the amygdala and hippocampus, where they may be involved in short-term memory and emotion. ERβ has also been identified in the cerebellum and in cortical regions.

The article by Ogawa and colleagues in this issue of PNAS (16) deals with the effect on male sexual behavior of targeted disruption of both ERα and ERβ in mice (the $\alpha\beta$ERKO mice). Previous studies by this group in mice that lack the gene for either ERα (αERKO) or ERβ (βERKO) individually have shown that male sexual behaviors are partially disrupted or virtually normal. αERKO mice, although they rarely ejaculated and were infertile, manifested almost normal frequency of mounts but reduced numbers of intromissions (17). In contrast, all three components of sexual behavior were present and robust in βERKO males (18). However, in ArKO male mice, sexual behavior is severely compromised, including a marked reduction in mount frequency and prolonged mount latency (ref. 5; K. M. Robertson and M. E. E. Jones, personal communication; Table 1). However male ArKO mice are fertile at least until the age of 14–20 weeks (9). No detailed analysis of ArKO ejaculatory or intromission behavior has yet been reported. These results suggest either that ER activation may be only partially responsible for the induction of male sexual behavior or else in αERKO and βERKO mice the one missing ER gene is compensated for by the other.

In contrast to sexual behavior, aggressive behavior is greatly reduced in αERKO male mice (16, 19). In particular, male typical offensive attacks were almost abolished in both gonadally intact and testosterone-treated gonadectomized αERKO mice, whereas lunge and bite attacks, which are considered to represent mild and short-lasting aggressive behaviors, were still present. In βERKO male mice on the other hand, aggressive behavior was not reduced but rather was elevated, depending on age and social experience (17).§ These findings suggest that ERβ might be inhibitory of male aggressive behavior, which is facilitated by action of the androgen receptor, ERα, or both. So again the question arises, How might the deletion of both ERα and ERβ gene function affect male aggressive behavior? To answer these questions, Ogawa *et al.* (16) used double-knockout mice lacking both ERα and ERβ genes, namely the $\alpha\beta$ERKO mice. Aggressive behaviors and sexual behaviors were evaluated in male $\alpha\beta$ERKO mice, and results were compared with those of single-knockout (αERKO and βERKO) as well as wild-type littermates. Sexual behavior was studied in the male mice by placing a female in the male's own cage. The females were ovariectomized and injected s.c. with estradiol benzoate and progesterone to ensure high sexual receptivity. For

See companion article on page 14737.

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Table 1. Mounting behavior of ArKO and wild-type male mice

Mice	No. of mounts/ 20 min	Time to first mount, min	Mount latency, min
Wild type	11 ± 1.9	4.41 ± 1.3	1.37 ± 0.02
ArKO	0	—	—

Sexual behavior appeared normal in the wild-type male animals at age 12–14 weeks. In contrast, ArKO mice demonstrated severely impaired mounting behavior (K. M. Robertson and M. E. E. Jones, personal communication).

each male the latency and number of attempted mounts, intromissions, and ejaculations were recorded. Ultrasonic vocalization was also monitored for the first 10 min after introduction of the female mouse. Male aggressiveness was evaluated by using two paradigms—the resident intruder paradigm and the homogeneous set paradigm. For the former, each male was tested in its home cage against a group-housed intruder mouse that had been subjected to olfactory bulbectomy. For each experimental male, latency to the first aggressive act, cumulative duration of all aggressive bouts, number of lunge/bite aggression bouts, number of offensive attack bites, and cumulative duration of sexual behavior by resident mice toward intruder mice, that is to say chasing with attempted mounts, were recorded. For the homogeneous set aggression test, pairs of body weight-matched males from the same genotype were placed on either side of the test cage which was divided in the center by a transparent acrylic board. After a 5-min adaptation period, the divider was removed and aggressive behavior was recorded for 15 min. For each pair, numbers of tail rattling, numbers of pairs showing aggressive behaviors, the latency to the first aggressive act, cumulated duration of all aggressive bouts, number of pairs showing sexual behaviors, and cumulated duration of sexual behavior were all recorded.

The results indicate that male sexual behavior was completely disrupted in $\alpha\beta$ERKO males. These animals exhibited neither mounts, intromissions, nor ejaculations. The other three mouse genotypes—namely, αERKO, βERKO, and the wild type—exhibited sexual behaviors typical of each genotype as originally reported. Ultrasonic vocalization, which normally accompanies such social investigation as licking of the female genital area or sexual chasing, was also severely compromised. On the other hand, the βERKOs, the wild type, and the αERKOs vocalized normally. Taken together, these findings indicate that male sexual responses to receptive females are completely disrupted in $\alpha\beta$ERKO males. Behaviorally, αERKO and $\alpha\beta$ERKO mice resembled each other only in terms of lack of ejaculation, whereas sexual behavior of βERKO males was not deficient. The observed mounting behavior of the $\alpha\beta$ERKO mice is similar to what has been previously reported for ArKO—i.e., aromatase-deficient—mice (ref. 5; Table 1). By comparison with these observations on mice, estrogen treatment of one of the men with aromatase deficiency resulted in increased libido, frequency of intercourse, masturbation, and erotic fantasies (20).

By contrast, male aggressive behavior of the $\alpha\beta$ERKO mice was very similar to that of the αERKO mice, namely that $\alpha\beta$ERKO males rarely showed offensive attacks in all four tests. Lunge and bite attacks occurred, which are believed to be defensive type aggression most typically seen in pregnant mice. On the other hand, in the wild-type and βERKO mice substantial numbers of offensive attacks were observed. As previously reported,[§] βERKO mice tended to be more aggressive than wild-type controls. Although a detailed analysis of the aggression behavior of ArKO mice has not been reported, anecdotally they have also been reported to show little aggressive behavior and thus in this respect appear to be similar to the $\alpha\beta$ERKO or the αERKO mice (K. M. Robertson and M. E. E. Jones, personal communication).

Taken together, abolition of estrogen representation, whether it be by knockout of both ERα and β receptors or knockout of the gene encoding aromatase, results in loss of male sexual behavior, whereas abolition of the function of either of the estrogen receptors ERα or ERβ individually does not do this. Thus the presence of either one of the ERs is sufficient for the expression of simple mounting behavior in male mice, indicative of a redundancy in function. In other words, in this capacity, the two ERs can complement each other. This appears to be the first example of such complementarity, but no doubt other examples will be reported in the future.

However, the same cannot be said for male aggressive behavior. The present results suggest that the induction of offensive attacks, unlike male sexual behavior, may be regulated predominantly in a facilitatory manner by the activation of ERα. That βERKO mice are significantly more aggressive than wild-type controls suggests that these behavioral changes may be due to lack of an inhibitory action by ERβ on ERα-mediated functions in the βERKO mice. Such an action could stem from areas of the brain exhibiting core expression of the two ERs or else from independent ERβ- and ERα-regulated pathways that converge on a common neural pathway mediating aggressive behavior responses. Recent *in vitro* studies have provided evidence that the ERβ can indeed modulate ERα transcriptional activity (21, 22). Whereas both ERα and ERβ contain a functional AF2 transactivation domain, ERβ does not contain a strong AF1 within its amino terminus but rather contains a repressor domain (21). When both ERβ and ERα were cotransfected into cells together with an estrogen-responsive reporter gene construct, it was determined that ERβ functions as a transdominant inhibitor of ERα transcriptional activity and that ERβ decreases overall cellular sensitivity to estradiol (21, 22). This is believed to be due to the formation of heterodimers between ERα and ERβ within target cells. Thus in areas of the brain where both ERα and ERβ are expressed within the same cell, ERβ could act to directly inhibit ERα transcriptional activity and thus the relative expression levels of the two isoforms would be a key determinant of cellular responses to agonist and antagonists. The male aggression behavior of the ER knockout models described by Ogawa *et al*. may be indicative of the physiological relevance of these *in vitro* findings.

These results point to an important role for estrogens in both male sexual behavior and male aggression behavior. A significant question then relates to the origin of the estrogen, which is required for both the establishment and the maintenance of these behavior patterns. As indicated previously, in both postmenopausal women and men, estrogen ceases to be of significance as a circulating hormone—rather, estradiol is produced in a number of extragonadal sites, where it acts in a paracrine and/or intracrine fashion. This includes the brain, where aromatase expression is found in numerous sites. Consistent with the role of local aromatase expression within the brain to provide the source of ligand for the brain estrogen receptors is the fact that the concentrations of estradiol in the hypothalamic and preoptic area are at least 10-fold greater than those in the circulation of both postmenopausal women and men (23) and are sufficient to transactivate the ERs.

The mechanisms whereby estrogen affects brain function are only now beginning to be investigated. For example the serotonin 2A receptor (5-HT2AR), which is a target for LSD (lysergic acid diethyl amide) and antipsychotics such as clozapine, is expressed in the cell bodies of the dorsal raphe, whose serotonergic fibers innervate many sites, including the frontal cortex, cingulate cortex, piriform cortex, olfactory tubercle, and striatum. Estrogen increases the receptor concentration in all of these areas, and this action is blocked by

tamoxifen (23). Serotonin transporters (SERTs) are also expressed in cells of the dorsal raphe. Both the 5-HT2ARs and SERTs are decreased after castration in males, and their levels are restored by treatment with testosterone or estradiol, but not by dihydrotestosterone (24). It should be noted that the patterns of expression of ERβ and aromatase in the brain, but not that of ERα, follow quite closely the pattern of 5-HT2ARs (14, 25). In this context it is well known that selective serotonin reuptake inhibitors (SSRIs) such as fluoxetine have an inhibitory effect on sexual function, including libido.

Because circulating levels of estrogens in men and postmenopausal women are very low, and production of ligand for ERs within the brain appears to depend primarily on local aromatase activity, then it would appear that in these populations circulating levels of estrogen have little relevance in this context. Indeed, they would appear to be merely a reflection of the estrogen that spills over into the circulation from local sites of production and is not immediately metabolized therein. By contrast, aromatase in brain depends on an external source of C_{19} precursor, namely testosterone. In the male circulation, the levels of testosterone are more than an order of magnitude greater than those circulating in the plasma of postmenopausal women. Although testosterone levels in the plasma of men decrease with advancing years, compared with women men maintain a much higher circulating level of precursor testosterone throughout life which is available, therefore, for conversion to estradiol in extragonadal sites, including the brain. Thus the uninterrupted sufficiency of circulating testosterone in men throughout life supports the local production of estradiol by aromatization of testosterone in estrogen-dependent tissues, including the brain, and thus may afford ongoing protection against the so-called estrogen deficiency diseases (10). This may be important in terms of protecting the bones of men against mineral loss and might contribute to the maintenance of cognitive function and prevention of Alzheimer's disease in men. The use of testosterone as a component of hormone replacement therapy for postmenopausal women who complain of loss of libido is increasingly becoming accepted (26). Currently there is also considerable interest in the role of testosterone, perhaps acting as a precursor of estrogen, in cognition and mood. Results presented in the study of Ogawa *et al.* (16) strongly support the concept that this as well as other roles of testosterone is mediated, at least in part, by its conversion in the brain to estradiol, which subsequently acts as a ligand for either of the ER isoforms.

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